REMARKS

The Examiner has indicated that the amendments submitted on May 2, 2003 and have been entered. Applicants appreciatively note that the Examiner has indicated Claims 20-23 are allowed. Applicants also note that Claims 19 and 29-30 are only objected to and not rejected. Applicant has added new Claims 33-39, in which the elements include the testing of peptide fragments of a protein (*i.e.*, the entire protein is not tested as a whole, rather peptide fragments of the protein are tested). These new Claims also recite the use of specific cytokines for differentiating the dendritic cells. These new Claims are included to more specifically recite particular embodiments of the present invention. Applicants respectfully submit that there is more than ample support in the Specification as filed for these new Claims and no new matter is included therein.

Applicants appreciate the Examiner's removal of his previous rejections. Claims 17 and 18 stand rejected under 35 U.S.C. §103(a), as allegedly being unobvious under Garman et al. (US Patent No. 5,820,862), in view of Macatonia *et al.*, Mehta-Damani *et al.*, or Takamizawa *et al.*

The Examiner indicates that the Garman *et al.* reference teaches identification of T-cell epitopes within a protein allergen and modification thereof. However, the Examiner admits that the Garman *et al.* reference fails to teach the use of naïve T-cells and that Garman *et al.* teach epitope screening with T-cells from sensitized individuals (Office Action, page 2).

The Examiner further argues that each of the secondary references teaches that it is possible to obtain human blood samples and derive dendritic cells (DCs) and naïve T-cells from them, such that the DCs can present antigen to the naïve T-cells to induce a proliferative response. The Examiner alleges that it would have been obvious to identify epitopes within the allergen of Garman *et al.* by using DCs and naïve T-cells from a blood sample as taught by the secondary references. The Examiner argues that the motivation to do so would have been to conduct tests using blood cells from non-sensitized individuals, so that one would not need to find patients with the allergic disorder.

The Examiner also refers to his previous rejection and argues that the amendments filed with the RCE necessitated this rejection, as the Claims have been

amended such that Applicants have not overcome the prior art of record. Applicants must respectfully disagree.

Nonetheless, in order to further their business interests and the prosecution of the present application, yet without acquiescing to the Examiner's arguments, Applicants have amended Claim 17 to more clearly recite that the dendritic cells are differentiated in the presence of cytokines. Applicants submit that these amendments find more than sufficient support in the Specification. Applicants further submit that these amendments do not render the present Claims anticipated nor obvious under any of the cited references. Likewise, Applicants submit that newly added Claims 33-39 are unobvious over the cited art, as these Claims incorporate the use of specific cytokines and further include the element of peptide fragments. As indicated above, these new Claims find more than sufficient support in the Specification as filed and do not add any new matter. The Examiner's rejections are addressed in greater detail below.

A. There is No *Prima Facie* Case Of Obviousness

A *prima facie* case of obviousness requires the Examiner to cite to a combination of references which (a) suggests or motivates one of skill in the art to modify their teachings to yield the claimed invention, (b) discloses the elements of the claimed invention, **and** (c) provides a reasonable expectation of success should the claimed invention be carried out. Failure to establish **any** one of these requirements precludes a finding of a *prima facie* case of obviousness and, without more, entitles Applicants to withdrawal of the rejection of the claims in issue.[2] Applicants urge that the Examiner has failed to establish not one, but **all three** requirements as discussed below.

1. The Combined References Fail to Disclose All of the Claim Limitations

It is axiomatic for establishing a *prima facie* case of obviousness that "all the claim limitations must be taught or suggested by the prior art."[3] However, the Examiner has not established that the combined references disclose the all of the limitations set forth in the pending Claims, including obtaining human dendritic and naïve CD4+ and/or

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2 *See e.g., Northern Telecom Inc. v. Datapoint Corp.*, 15 USPQ2d 1321, 1323 (Fed. Cir. 1990); and *In re Dow Chemical Co.*, 837 F.2d 469, 5 USPQ2d 1529 (Fed. Cir. 1988).

3 MPEP § 2143.03, citing *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974).

CD8+ cells from a single source, differentiating the dendritic cells in the presence of cytokines, combining the differentiated dendritic cells with the CD4+ and/or CD8+ cells, and a peptide of interest. Indeed, Applicants respectfully submit that none of the requirements for a showing of obviousness has been made. To the contrary, the references cited by the Examiner highlight the unobvious nature of the presently claimed invention.

a. The Garman *et al.* Reference Fails To Disclose All of the Claim Limitations

With respect to each of the rejected Claims 17-18, Applicants previously argued that this reference fails to teach the use of naïve T-cells, a fact with which the Examiner agrees (Office Action, page 2). Likewise, new Claims 33-39 are not taught nor suggested by the Garman *et al.* reference. Thus, the primary reference does not teach each limitation of the pending Claims.

b. The Macatonia *et al.* Reference Does Not Provide The Elements Which Are Absent From Garman *et al.*

Applicants respectfully submit that the Macatonia *et al.* reference does not bridge the gaps of Garman *et al.* For example, neither this reference, nor Garman *et al.* teach the limitation of differentiating the DCs prior to their combination with T-cells. In contrast to the presently claimed invention, the Macatonia *et al.* reference indicates that the DCs are pulsed with HIV, HIV peptides or recombinant gp120 (*See*, page 401) and are then combined with T-cells. The presently claimed method involves differentiating the DCs by exposing them to cytokines (*See e.g.*, page 26, lines 1-11), prior to combining them with T-cells. There is no suggestion in either the Macatonia *et al.*, reference, or the Garman *et al.* reference that such a differentiation step be conducted. Thus, these references, taken alone or in combination do NOT teach nor even suggest the presently claimed invention.

c. The Mehta-Damani *et al.* Reference Fails To Supplement The Missing Limitations

Applicants also respectfully submit that the Mehta-Damani *et al.* reference does not teach all of the limitations of the pending Claims. Mehta-Damani *et al.* teach an *in vitro* system for generating antigen-specific CD4+ T-cells from previously umprimed individuals. As described in this reference, the method utilizes DCs (and macrophages, in some embodiments) and T-cells isolated from human peripheral blood (*See*, page 1207). As with the Garman *et al.* and Macatonia *et al.* references, but unlike the presently claimed invention, the method of Mehta-Damani does not involve a step in which the DCs are differentiated in the presence of cytokines. Rather, the purified DCs obtained from human peripheral blood are irradiated and then added to CD4+ T-cells in culture. Thus, there is no teaching in Mehta-Damani that would motivate one of skill in the art to treat the DCs so as to promote their differentiation, prior to their use as antigen presenting cells for T-cells. Therefore, Applicants respectfully submit that this reference, taken alone, in combination with Garman, or in combination with any of the other cited references, does not teach nor motivate one to produce the presently claimed invention.

d. The Takamizawa *et al.* Reference Fails to Provide the Missing Elements of the Pending Claims

As with the Garman *et al.*, Macatonia *et al.*, and Mehta-Damani *et al.* references, Applicants respectfully submit that the Takamizawa *et al.* reference does not teach the presently claimed invention, either alone or in combination with any of the other references. Takamizawa *et al.* teach the use of two populations of DCs to present antigens to T-cells. Their data indicate that "differentiated DC can be obtained from a population of HLA-DR^{bright}, lineage-negative, CD2^{+} cells present in peripheral blood, and that the DC derived from these DCp (DC precursors), but not DC derived from precursors that lack CD2 expression (CD2^{-} DCp), present nominal antigens to naïve T cells." (Takamizawa *et al.*, at page 2134). In this reference, the DC precursors were differentiated by incubating the precursor cells (depleted of monocytes) in conditioned medium from PHA-activated peripheral blood mononuclear cells (PBMC). There is no teaching of differentiating DCs in the presence of cytokines (*e.g.*, GM-CSF, IL-4, TNFα,

nor IL-1α as recited in the newly added Claims. Takamizawa *et al.* only teaches the use of medium conditioned by incubation of PBMC stimulated by PHA.

Furthermore, Takamizawa *et al.* do NOT teach a method that involves the use of peptides to stimulate T-cells. Rather, Takamizawa *et al.* teach the use of KLH or HIV gp160 antigens, which are clearly not "peptides." There is no indication that the use of peptides or peptide fragments of proteins, rather than these large molecules, would successfully result in a T-cell response in the presence of DCs. Thus, Takamizawa *et al.* do not teach the presently claimed invention, in which peptides or peptide fragments are used in conjunction with differentiated DCs to induce a T-cell response. Thus, Takamizawa *et al.*, taken alone or in combination with any of the above references does not teach the presently claimed invention. Indeed, Applicants submit that particularly when viewed as a whole, the presently claimed invention is unobvious over the prior art[4]. In addition, Applicants respectfully submit that as indicated in *Intel Corp. v. United States Int'l Trade Comm'n*, 946 F.2d 821, 842, 20 USPQ2d 1161, 1179 (Fed. Cir. 1991):

> Claim limitations may, and often do, read on the prior art, particularly in combination patents. That all elements of an invention may have been old (the normal situation), or some old and some new, or all new, is however, simply irrelevant. Virtually all inventions are combinations and virtually all are combinations of old elements. [Quoting from *Environmental Designs, Ltd. v. Union Oil Co.*, 713 F.2d 693, 698, 218 USPQ 865, 870 (Fed. Cir. 1983)].

Thus, *even if* (which Applicants submit they are not) the elements of the presently claimed invention are described in the prior art, Applicants respectfully submit that the invention itself is not described, taught, nor suggested in any of the cited references, taken alone or in any combination. Thus, Applicants respectfully submit that this rejection should be withdrawn.

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[4] "[T]he question under 35 U.S.C. § 103 is not whether the differences *themselves* would have been obvious. Consideration of differences, like each of the findings set forth in *Graham*, is but an aid in reaching the ultimate determination of whether the claimed invention *as a whole* would have been obvious." *Stratoflex Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1537, 218 USPQ 871 (Fed. Cir. 1983) (*emphasis original*).

2. Motivation To Practice The Recited Combination Of Steps

An essential requirement for a *prima facie* case of obviousness is whether a person skilled in the art would be **motivated** to modify the reference to arrive at the **claimed invention.**[5] In particular,

> "the examiner must show *reasons* that the skilled artisan, confronted with the same problems as the inventor and with no knowledge of the *claimed invention*, would select the elements from the cited prior art references for combination in the manner claimed."[6]

Such evidence is lacking in the present Office Action and well as the previous Office Action referred to by the Examiner (paper 30), as none of the references, taken alone or in combination suggest using cytokines to differentiate the DCs prior to the combination of the DCs with the T-cells, as presently claimed. The Examiner has provided no references nor reasons why one of skill in the art would take the cited combination of references, and then modify the methods so as to differentiate the DCs using cytokines prior to their use in an assay system for methods for determining T-cell epitopes or reducing the allergenicity of proteins, as presently claimed. Nor does the Examiner point to any reference that teaches the combination of differentiated DCs and exposure of CD4+ and/or CD8+ T-cells to peptides or peptide fragments in the presence of differentiated DCs, such that the claimed methods for determining a T-cell epitope of a peptide, nor methods for reducing the allergenicity of proteins are produced. Indeed, Applicants submit that there is nothing in the prior art to lead a person of ordinary skill to the combination of the teachings of the these references to design the claimed methods of the present invention other than the hindsight knowledge of Applicants' methods. Furthermore, "[T]he motivation to combine references cannot come from the invention itself." (*Heidelberger Druckmaschinen v. Hantscho Commercial Products*, 30 USPQ2d 1377, 1380, 21 F.3d 1068 (Fed. Cir. 1994)). Thus, Applicants respectfully submit that this prong of the obviousness analysis is not met and request that this rejection be withdrawn.

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5 *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598-99 (Fed.Cir. 1988); and *In re Jones*, 21 USPQ2d 1941, 1943 (Fed. Cir. 1992).

6 *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998); *Robotic Vision Systems Inc. v. View Engineering Inc.*, 51 USPQ2d 1948 (Fed. Cir. 1999), (*emphasis added*).

3. A Reasonable Expectation Of Success Is Not Established

A further fundamental requisite of establishing a *prima facie* case of obviousness is that there is a reasonable expectation of success in practicing the recited method steps or producing the recited compositions.

> "[T]he reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure."[7]

There is no indication in the references cited nor the Examiner's arguments, that the cited references, taken alone or in combination, would successfully produce the presently claimed invention. As indicated above, Applicants believe that the only way to obtain the presently claimed invention would be to use the hindsight provided by the present Specification itself. This is not permissible[8].

Indeed, "[o]ne cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." (See *In re Fine*, 837 F.2d 1071, 1075, 5 USPQ2d 1596 (Fed. Cir. 1988)). As there is no expectation of success that the combination of the teachings of the cited references would result in the presently claimed invention, this prong of the obviousness analysis is not met. Thus, Applicants respectfully request that this rejection be withdrawn.

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7 *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988), as cited in *In re Vaeck*, 20 USPQ2d 1438, 1442 (Fed. Cir. 1991).

8 *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992); *See also, W. L. Gore & Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303, 311 (Fed. Cir. 1983) ("To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher.").

CONCLUSION

As all of the Examiner's rejections and arguments have been herein addressed and In light of the above remarks, the Applicants respectfully submit that the pending claims are in condition for allowance. Thus, Applicants respectfully request that a Notice of Allowance be issued at an early date. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (650) 846-5838.

Respectfully submitted,

Date: September 25, 2003

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APPENDIX II
CLEAN VERSION OF THE ENTIRE SET OF PENDING CLAIMS AS AMENDED IN THIS COMMUNICATION

The following is a list of the Claims as they would appear following entry of this amendment.

17. (Currently Amended) A method for determining a T-cell epitope of a peptide, comprising the steps of:

(a) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

(b) differentiating said dendritic cells in the presence of cytokines;

(c) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with the peptide, said peptide comprising said T-cell epitope; and

(d) measuring proliferation of said T-cells in said step (c).

18. (Currently Amended) A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell that has been differentiated by exposing said dendritic cell to cytokines, with a peptide comprising said T-cell epitope; and

(ii) contacting said dendritic cell and peptide with a naïve T-cell, wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

19. (Previously Added) The method according to claim 18, wherein the protein is a protease.

20. (Previously Amended) A method for reducing the allergenicity of a microbial subtilisin comprising the steps of:

(a) determining a T-cell epitope of said subtilisin comprising (i) obtaining from a from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells; (ii) promoting differentiation in said solution of dendritic cells; combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with peptide fragments of said subtilisin; and (iv) measuring proliferation of said T-cells in said step (iii); and

(b) modifying the peptide which includes the T-cell epitope to neutralize said epitope.

21. (Previously Added) The method according to claim 20, wherein the microbial subtilisin is derived from a *Bacillus*.

22. (Previously Added) The method according to claim 21, wherein the *Bacillus* is selected from the group consisting of *B. lentus, B. subtilisin, B. amyloliquefaciens* and *B. licheniformis*.

23. (Previously Amended) The method according to claim 20, wherein said epitope of said microbial subtilisin is modified by: (a) substituting the amino acid sequence of the epitope with an analogous sequence from a human homolog of said microbial subtilisin; (b) substituting the amino acid sequence of the epitope with an analogous sequence from a non-human homolog of said microbial subtilisin; or (c) substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

29. (Previously Added) The method according to claim 18, wherein said T-cell epitope is modified by a substitution selected from the group consisting of:

(a) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a human homolog to the protein of interest;

(b) substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest; or

(c) substituting the amino acid sequence of said T-cell epitope with a sequence which substantially mimics the major tertiary structure attributes of the epitope.

30. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the T-cell epitope with an analogous sequence from a human homolog to the protein of interest.

31. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of said T-cell epitope with an analogous sequence from a non-human homolog to the protein of interest.

32. (Previously Added) The method according to claim 29, wherein said T-cell epitope is modified by substituting the amino acid sequence of the epitope with a sequence which substantially mimics the major tertiary structure attributes of said T-cell epitope.

33. (New) A method for determining a T-cell epitope of a protein, comprising the steps of:

(a) obtaining a protein and preparing peptide fragments of said protein;

(b) obtaining from a single human blood source a solution of dendritic cells and a solution of naïve CD4+ and/or CD8+ T-cells;

(c) promoting differentiation of said dendritic cells by exposing said dendritic cells to granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4);

(d) combining said solution of differentiated dendritic cells and said naïve CD4+ and/or CD8+ T-cells with said peptide fragments, wherein said peptide fragments comprise said T-cell epitope; and

(e) measuring proliferation of said T-cells in said step (d).

34. (New) The method of Claim 33, wherein said promoting differentiation of said dendritic cells in step (c) further comprises exposing said dendritic cells to tumor necrosis factor alpha (TNF-α).

35. (New) The method of Claim 33, wherein said promoting differentiation of said dendritic cells in step (c) further comprises exposing said dendritic cells to interleukin-1 alpha (IL-1α).

36. (New) A method of reducing the allergenicity of a protein comprising the steps of:

(a) identifying a T-cell epitope in said protein by

(i) contacting an adherent monocyte-derived dendritic cell that has been differentiated in the presence granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4), with a peptide comprising said T-cell epitope; and

(ii) contacting said differentiated dendritic cell and peptide with a naïve T-cell, wherein said naïve T-cell has been obtained from the same source as said adherent monocyte-derived dendritic cell, and whereby said T-cell proliferates in response to said peptide; and

(b) modifying said protein to neutralize said T-cell epitope such that the modified protein induces less than or substantially equal the baseline proliferation of said naïve T-cells.

37. (New) The method of Claim 36, wherein said contacting said monocyte-derived dendritic cell in step (a)(i) further comprises exposing said dendritic cell to tumor necrosis factor alpha (TNF-α).

38. (New) The method of Claim 36, wherein said contacting said monocyte-derived dendritic cell in step (a)(i) further comprises exposing said dendritic cells to interleukin-1 alpha (IL-1α).

39. (Previously Added) The method of Claim 36, wherein said protein is a protease.